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Sargeant, A.J.; de Haan, A.

#### ***published in***

Journal of Physiology and Pharmacology  
2006

#### ***document version***

Publisher's PDF, also known as Version of record

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#### ***citation for published version (APA)***

Sargeant, A. J., & de Haan, A. (2006). Human muscle fatigue: the significance of muscle fibre type variability studied using a micro-dissection approach. *Journal of Physiology and Pharmacology*, 57, 5-16.

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A.J. SARGEANT, A. DE HAAN

## HUMAN MUSCLE FATIGUE: THE SIGNIFICANCE OF MUSCLE FIBRE TYPE VARIABILITY STUDIED USING A MICRO-DISSECTION APPROACH

Institute for Biophysical and Clinical Research into Human Movement,  
Manchester Metropolitan University, Manchester, U.K. and Institute for Fundamental  
and Clinical Studies of Human Movement, Vrije University, Amsterdam, The Netherlands.

During human locomotion the ability to generate and sustain mechanical power output is dependent on the organised variability in contractile and metabolic properties of the muscle fibres that comprise the active muscles. In studies of human exercise we have used a micro-dissection technique to obtain fragments of single muscle fibres from needle biopsies before and after exercise. Each fibre fragment is divided into two parts. One part is used to characterize the fibre type in respect of the heavy chain myosin isoform expressed. The other part of the fragment is analysed for high energy phosphate concentrations. Fibres are classified on the basis of expressing either type I, type IIA, or type IIX myosin heavy chain isoforms. It should be noted however that in the type II population many fibres co-express both IIA and the IIX isoforms and we therefore characterize these fibres on the basis of the degree of co-expression. We have used this technique to examine the time course of high energy phosphate concentration and fatigue in different fibre populations during exercise. The progressive reduction of power during maximal sprint efforts may be interpreted as the cumulative effect of metabolic depletion in successive fibre type populations from IIX to IIXa to IIAx to IIA to I. One important application of the micro-dissection technique is that PCr content may also be used as a very sensitive metabolic marker for fibre type recruitment during very short duration concentric, isometric and eccentric exercise.

**Key words:** *muscle power, myosin heavy chain isoforms, phosphocreatine-to-creatine ratio, single fibers*

## INTRODUCTION

It is the ability to generate muscle power and sustain power output that enables us to walk and run, jump and climb. It is as important to an elderly person who wants to walk to the shops, or climb the stairs to go to bed, as it is for the Olympic Athlete or the prima ballerina. Movement is indeed "*the essence of the human machine*" and the ability to sustain movement, that is, resist fatigue is of critical importance.

There is a range of experimental models all of which can contribute to our understanding of the determinants and constraints of human muscle power. These range from *in vitro* studies of contractile protein motility and studies of single muscle fibre preparations; through studies of whole animal muscles *in vitro*, or *in situ*; to the measurement of performance in human whole body exercise. We review here an approach that we believe makes a useful contribution to the study of human power and fatigue, especially in the context of understanding the significance of the heterogeneity of contractile and metabolic properties of human muscle fibres.

In this 'top-down' approach we have sought to characterise the magnitude of fatigue during performance of a whole body locomotory task, and to interpret that in relation to the metabolic changes in a sample of human muscle obtained by needle biopsy in the resting and fatigued states and during recovery. Using ultra-sensitive techniques developed in our laboratories we are able to micro-dissect single fibre fragments and characterise part of each fragment using modified histochemical and electrophoretic techniques to determine fibre type. The remaining part of each characterised fragment is then used to determine changes in metabolite concentrations with HPLC.

*Muscle Power and fatigue in human exercise: the problem*

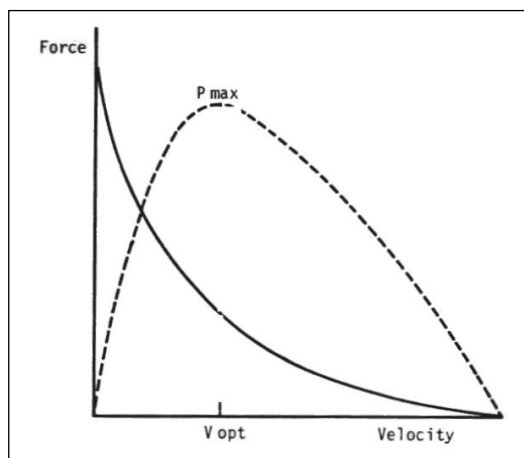
There is a fundamental difficulty in seeking to quantify the effect of fatigue on power output in whole body human exercise, and that is the nature of the force/velocity and therefore power/velocity relationship of muscle as shown in *Fig. 1*. Since fatigue in, for example, a locomotory task is often manifested and measured as a reduction in the speed of movement it will be obvious that any change in speed will also affect the intrinsic power available as determined by the power/velocity relationship. Thus the true magnitude of fatigue may be obscured (1 - 3) (*Fig. 1*).

*Isokinetic cycling as a solution: studies of human muscle power and fatigue*

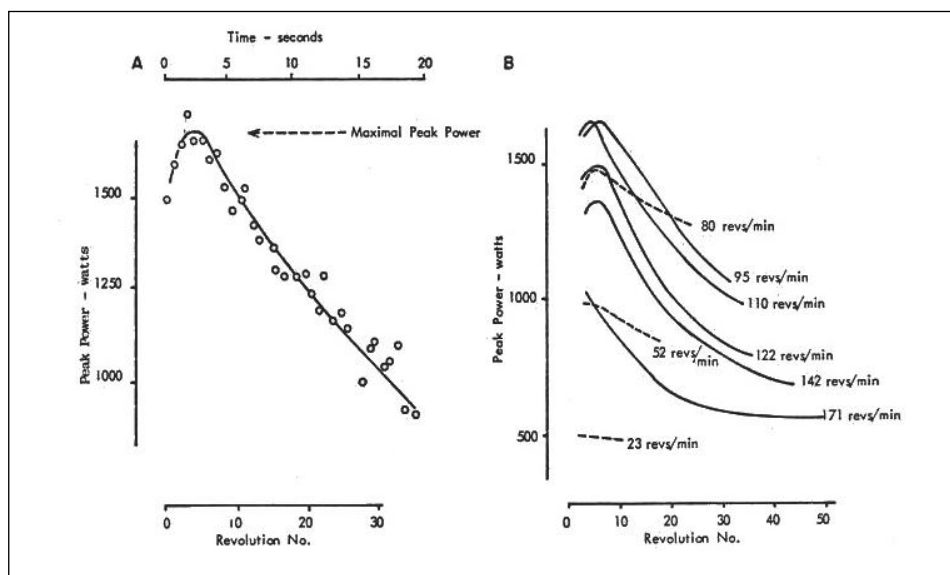
We therefore chose to study human locomotory performance in cycling using an isokinetic cycle ergometer (4). We were able to measure the power generated at a known constant pedalling rate during 20 seconds of maximum effort during which power typically declined by ~40% at 110 rev/min (*Fig. 2A*). Furthermore,

in a series of tests at different pedalling rates (*Fig. 2B*) we were able to characterise the maximum power/velocity (pedalling rate) relationship for cycling exercise and hence the optimum pedalling rate of  $\sim 120$  rev/min for maximum power output (*Fig. 3*).

In subsequent studies we measured the effect on maximum power of fatigue generated by sustained prior exercise of 6 minutes at  $\sim 90\%$  of  $\dot{V}_{O_{2\max}}$  (5, 6). In *Fig.*



*Fig. 1.* Schematic illustration of force velocity relationship of muscle as shown by the solid line. The mathematical consequence of which is that power in relation to velocity is of the form shown by the dashed line where maximum power ( $P_{\max}$ ) is reached at optimal velocity ( $V_{\text{opt}}$ ).



*Fig. 2.* A: typical changes for one subject in peak power during 20 second maximum effort at a constant pedalling rate of 110 rev/min on an isokinetic cycle ergometer. Data points are for each revolution. B: Changes in peak power in the same subject for 8 separate tests. Data points are omitted for clarity. Reprinted with permission from (4).

4B the data from a 25 sec maximum power test at 120 rev/min is shown under resting control and fatigued conditions. It can be seen that after fatiguing prior exercise the maximum power at the beginning of the test was reduced by ~25%. In marked contrast when the maximum power was measured at 60 rev/min the prior ‘fatiguing’ exercise had no effect (Fig. 4A). Summarising the data for five subjects at five pedalling rates it can be seen that the effect of the prior exercise

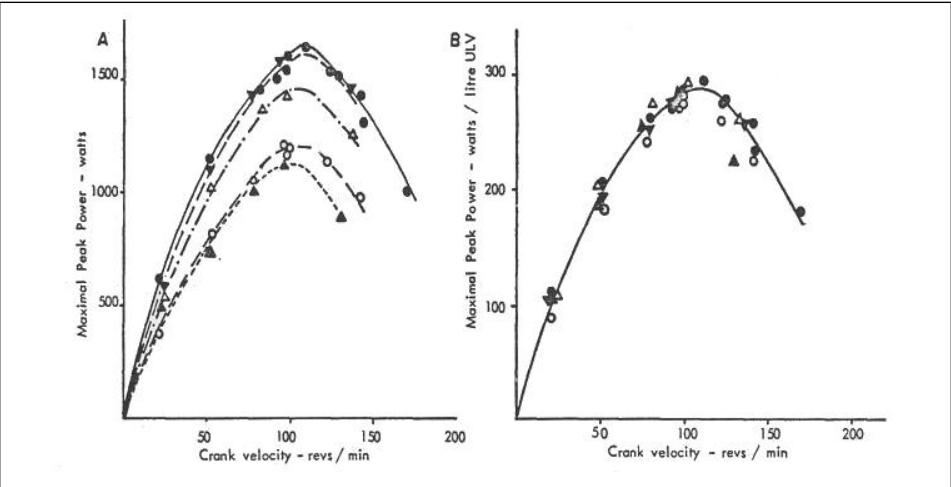


Fig. 3. A: Relationship of maximum peak power (reached at the beginning of each test) to the pedalling rate (revs/min) for 5 subjects.  
B: The same data as in panel A except that the maximum peak power has been normalized for the size of the active muscle mass. Reprinted with permission from (4).

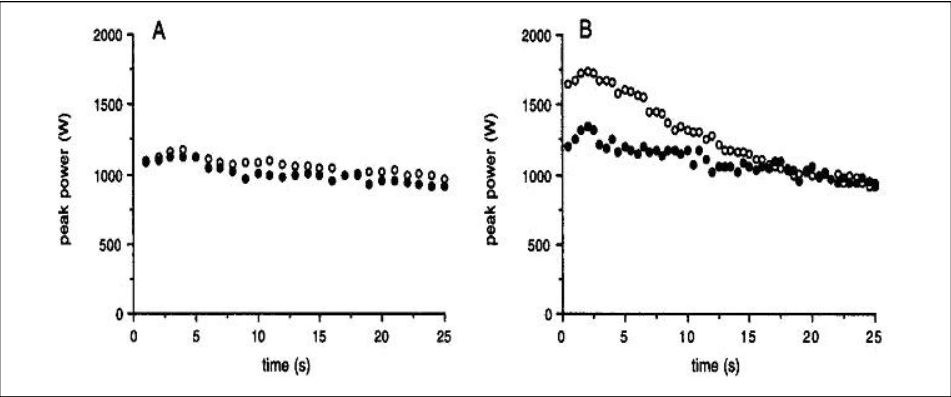
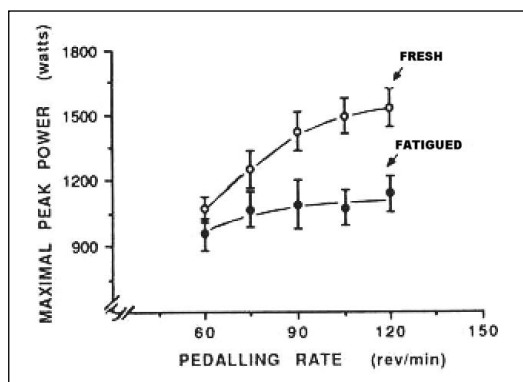
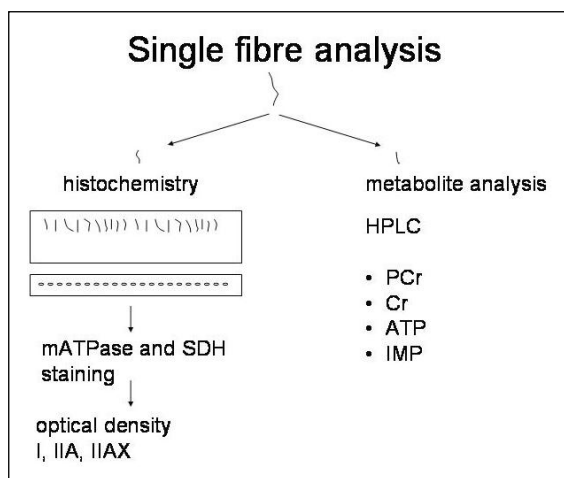


Fig. 4. Peak power generated by one subject during 25 maximal effort performed on the isokinetic cycle ergometer under rested control conditions (open circles) or following 6 minutes of prior fatiguing exercise performed at 90% of  $\dot{V}O_{2max}$  (filled circles). Data points are for each revolution in both conditions cycling at 60 revs/min pedal rate (panel A); and 120 revs/min (panel B). Reprinted with permission from (5).

was highly velocity dependent (*Fig. 5*). Paradoxically, however, *Fig. 4B* also shows that although at 120 rev/min the *maximum* power at the beginning of exercise was reduced as a consequence of the prior fatiguing exercise, the *rate* of fatigue was less when the muscle was already fatigued. Thus the power after 18 secs of maximum effort was the same in both the fatigued and control conditions. We suggested at the time that both the velocity dependent effect of fatigue on maximum power and the paradox of a lower rate of fatigue in the fatigued state could be explained by a selective fatigue of the faster more powerful fatigue sensitive fibres which might be expected to make a proportionately greater contribution to the whole muscle power as pedalling rate increased (1). Furthermore, because they are fatigue sensitive, they would already have been fatigued at the beginning of the 25 sec 120 rev/min exercise. In contrast, in the rested control condition the fatigue sensitive fibre population is still available to be fatigued and hence the higher rate in the first 18 seconds (6, 7).



*Fig. 5.* Group data for human maximal peak power cycling at 5 different pedalling rates in the rested control condition (open circles), and following 6 minutes of prior fatiguing exercise (closed circles). Mean (SE) data for 6 subjects. There was no significant effect of prior exercise at 60 or 80 revs/min but differences of ~25% were significant at the higher pedal rates demonstrating the velocity dependent effect of fatigue. Reprinted with permission from (5)



*Fig. 6.* Schematic to show the micro-dissection approach. A single fibre fragment is teased out from the biopsy and divided. The portion on the left is laid along with other fragments in a numbered sequence on a gelatin bed and covered with another layer of gelatin. The whole block is then rotated through 90 degrees for serial sectioning and histochemistry. The portion of fibre on the right is prepared and analysed for high energy phosphate content with HPLC as described in (8). Further fragments have also been analysed with SDS page.

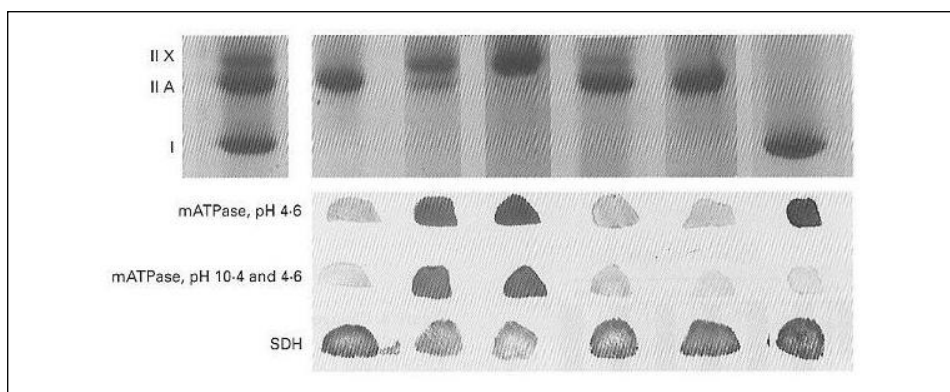
### *Micro-dissection and analysis of muscle biopsies*

In order to investigate the impact of selective fatigue of different muscle fibre populations on muscle power we developed a micro-dissection technique that would allow us 'to take the fatigued muscle apart' and determine the energetic status of the component muscle fibre populations. In these studies muscle biopsies obtained immediately after exercise were freeze dried and subject to micro-dissection and analysis as indicated in the schematic of *Fig. 6* (8, 9). On the left side of the schematic a portion of each fragment is subjected to histochemical and electrophoretic characterization as shown in *Fig. 7*, while the remainder of each fragment is analysed using HPLC to determine metabolite concentrations. *Fig. 8* shows the HPLC records for IMP and ATP and separately for PCr and Cr (for further explanation see (9)).

### *Human muscle power and selective fatigue of fibre populations*

We applied the micro-dissection technique in studies in which we asked subjects to perform maximum power output tests lasting 10 and 25 seconds pedalling at 120 revs/min. This exercise resulted in a 40% reduction in maximum power after 25 secs (10, 11). The data for [ATP] is summarised in *Fig. 9*. It can be seen that [ATP] in the type I fibre population is unchanged after 10 seconds and shows only a modest decrease after 25 secs. In type IIA fibres there is already a significant decrease to 60% of resting values after 10 secs and a further decrease to ~40% after 25 secs.

However, in those fast fatigue sensitive fibres expressing IIX myosin heavy chain isoform the [ATP] is reduced to ~30% of resting value after only 10 secs



*Fig. 7.* SDS-PAGE and histochemistry.

Histochemical and electrophoretic characterization of six single human skeletal muscle fibres. Fibres were classified (from left to right): type IIA, IIaX, IIX, IIaX, IIA and I (capital letters indicate predominance of one type of MyHC type. On the extreme left is a reference gel for whole muscle homogenate showing the position of the type I, IIA, and IIX isoforms. Reprinted from (11).

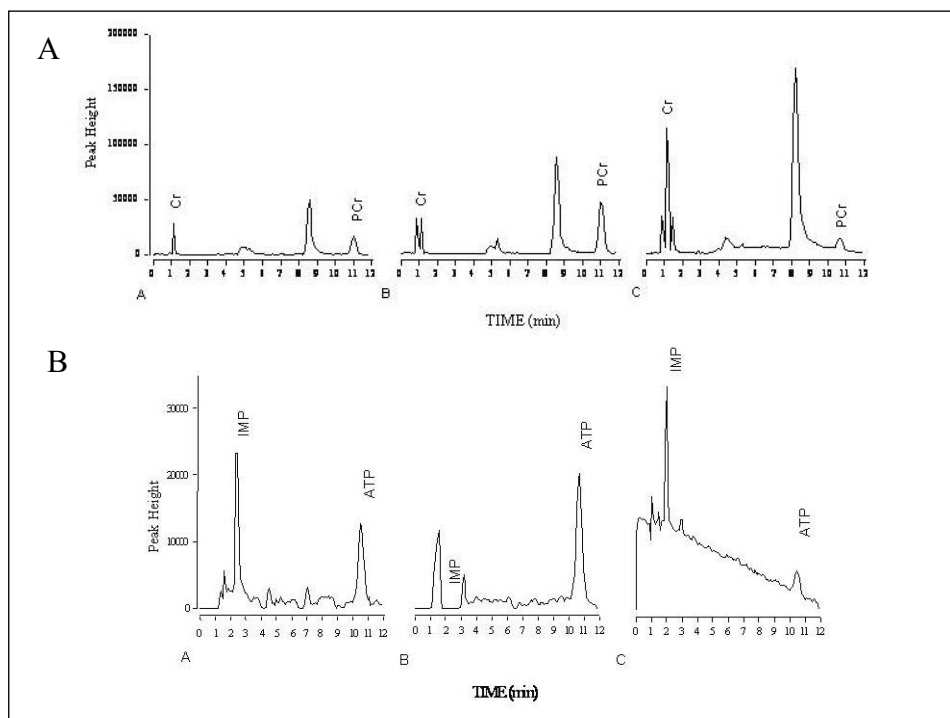


Fig. 8. A: shows the HPLC records for PCr and Cr from the standard (A), at rest (B) and following exercise (C).

B: shows the HPLC records and peaks for IMP and ATP again for the standard (A) and for rest (B) where no IMP is detectable, and post exercise (C). Reprinted from (9).

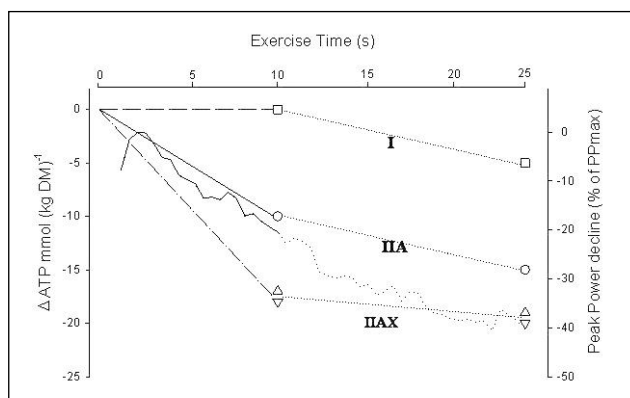


Fig. 9. Mean decline in [ATP] for type I, IIA, and IIXa (upright triangles) and IIXa (inverted triangles). Biopsies were obtained at 10 secs and 25 secs in separate experiments. A typical power profile is shown for one subject for the whole 25 secs. Reprinted from (10).

and remains at this level until the end of the 25 sec exercise. It can be seen that after 10 secs exercise there was already a 23% loss of power from the whole muscle and this was associated with almost maximal possible depletion of ATP in those fibres expressing some IIX MyHC isoform, suggesting that they probably



contribute little to the subsequent power and that a sequential metabolic challenge and failure of IIXa, to IIAx, to IIA, to I, underlies the whole-muscle fatigue seen in this type of maximal dynamic exercise. In these studies we have grouped the fibres according to proportion of co-expression of IIA and IIX isoforms. In fact there is a continuum of co-expression and therefore contractile and associated metabolic properties (8). Thus we would propose that a better representation of the change in [ATP] might be as shown schematically in Fig. 10 where a whole family of fibres co-expressing successively lower proportions of IIX MyHC isoform are shown from left to right with better preserved [ATP], and we would suggest, associated mechanical power output.

*Selective fatigue and selective recruitment – starting to put the picture together*

Of course it will be self evident that the extent to which any fibre population will be metabolically challenged and become fatigued will be a function both of

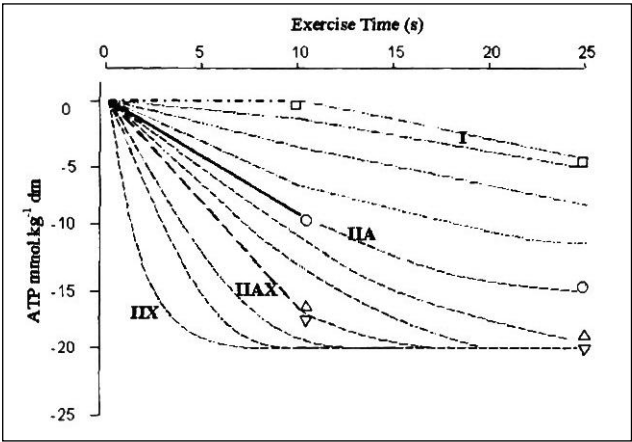


Fig. 10. Schematic suggestion of the probable decline in [ATP] for the continuum of fibre properties. Very few pure IIX fibres are seen in healthy adult muscle but if the IIAx fibres are already around the minimal possible levels after 10 secs it might be assumed that the IIX would be at that level even earlier. The actual data points from Fig. 9, are included as anchor points for the schematic.

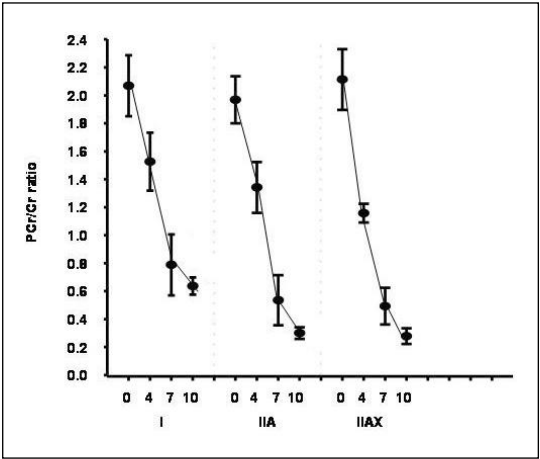


Fig. 11. Changes in phosphocreatine (PCr) content expressed as PCr/Cr ratio at rest and following 4, 7, and 10, maximal isometric contractions of the knee extensors. Drawn from data in (12).

the intrinsic fatigue sensitivity of that population of fibres in combination with the degree to which it is recruited during exercise. Our micro-dissection technique enables us to measure changes in phosphocreatine (PCr) as a sensitive indicator of fibre activity after a very few contractions (12). After only four 1 sec isometric contractions PCr was reduced to ~75, 65, and 53% of resting values in type I, IIA, and IIX fibres respectively, with further reductions after seven contractions to 38, 28, and 23% (Fig. 11).

We therefore used this technique to examine the metabolic activity of different fibre populations at different intensities of isometric contraction, viz, 39, 72, and 87% of MVC (13). As shown in figure 12, type I and IIA fibres showed the expected progressive involvement indicated by the significant leftwards shift of the cumulative distribution with increasing intensity of exercise. In contrast and rather surprisingly the IIX fibre population showed no evidence of metabolic involvement even at 72% of MVC. It was not until the very highest intensity (87%) that there was a reduction in PCr as indicated by a leftwards shift of the cumulative distribution.

This is somewhat surprising when one considers that in dynamic cycling exercise studies based on glycogen depletion suggest an involvement of all muscle fibre types at 90% of  $\dot{V}O_{2\max}$ , which is a level of exercise intensity that would only require approximately 40% of the maximum force generating capacity of muscle at the same velocity of contraction (14).

Subsequently we have been able to apply this technique to gain insight into the recruitment pattern during maximal lengthening, isometric and shortening

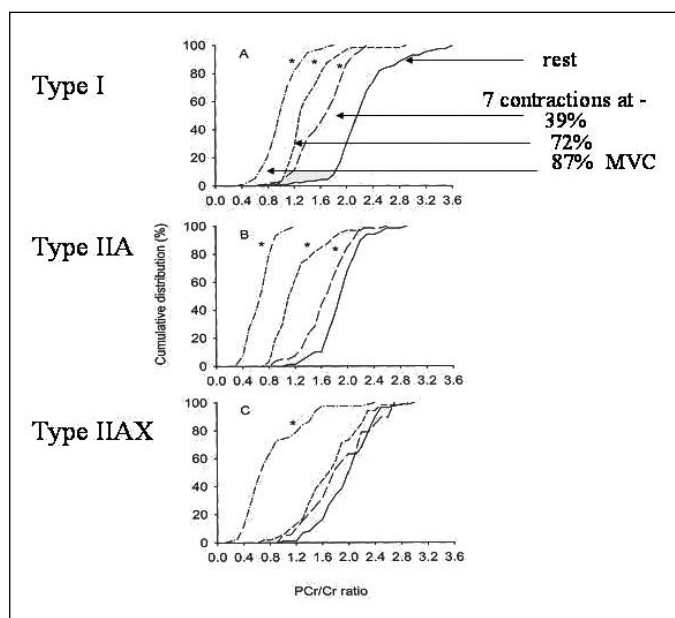
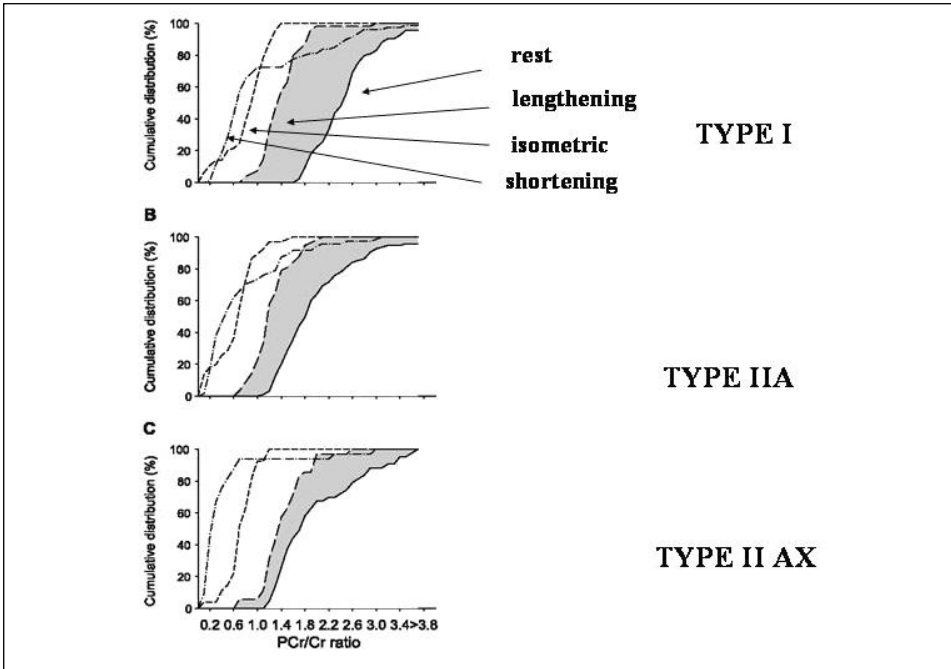


Fig. 12. Cumulative frequency distribution of single fibre PCr/Cr ratios in type I, IIA, and IIX fibres at rest and following seven isometric contractions at 39, 72, and 87% of MVC. In general the cumulative curves show a leftwards shift as intensity increases except in the case of the IIX fibres which are only active at an intensity beyond 72%. Reprinted from (13).



*Fig. 13.* Cumulative distribution of single fibre PCr/Cr ratios in type I, IIA and IIAX fibres at rest, and after a series of 10 lengthening (long dashed lines), isometric (short dashed lines), or shortening contractions (dash and dotted lines). The area between the cumulative distribution for rest and following lengthening contractions has been shaded to illustrate that far from there being a selective activation of type II fibres during lengthening contractions as is sometimes proposed the reverse seems to be the case in that the shift in the distribution is less marked in the type IIAX fibres (grey area) than in the type I and IIA. Adapted from (15).

contractions in human exercise (15). These data show no evidence of de-recruitment of type I fibres in lengthening contractions as has sometimes been proposed and show a progressive increase in PCr depletion in all fibre types from lengthening, to isometric, to shortening contractions reflecting increased metabolic turnover (*Fig. 13*). Adapted from (15).

## CONCLUSION

In conclusion our studies demonstrate the significance of the variability and continuum of muscle fibre contractile and metabolic properties in human mixed muscle for understanding the fatigue seen in whole body exercise, in which a profound loss of power may be attributable to selective fatigue of a relatively small population of fast fatigue sensitive fibres. To understand the underlying causes of fatigue it is necessary to integrate information about both the variability

of fibre properties and the pattern of recruitment in any particular type and intensity of exercise. We believe that our micro-dissection technique will prove a valuable and informative tool in the study of human muscle function and performance in health and disease.

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Received: October 13, 2006

Accepted: November 20, 2006

Author's address: Professor Anthony J. Sargeant, Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University Hassall Road, Alsager, ST7 2HL, UK. Phone Number: 44+(0)161 247 5591; e-mail: [a.j.sargeant@mmu.ac.uk](mailto:a.j.sargeant@mmu.ac.uk)